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NOTES

Susceptibility of *Bacteroides* spp. to Heavy Metals

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The susceptibility of 105 strains of *Bacteroides* to arsenate, silver, nickel, cobalt, lead, cadmium, chromium, and mercuric ions was tested by using an agar dilution technique. All strains of *Bacteroides* were multiply resistant. *Bacteroides fragilis* was significantly more resistant to silver ions than other *Bacteroides* species and slightly more resistant to several other ions. Arsenate was the most active ion tested, with 75% of strains being susceptible. All 25 strains of *B. fragilis* tested against silver sulfadiazine were susceptible.

There is little information on the susceptibility of *Bacteroides* spp. to heavy metals and metalloid compounds. Knowledge of this susceptibility may be of value in clinical situations and in providing functions for some of the cryptic plasmids which have been demonstrated in *Bacteroides* spp. (14, 18; T. V. Riley, unpublished data). In facultative anaerobes, plasmid-mediated resistance to heavy metals has been extensively studied (16), and comparative surveys in *Bacteroides* spp. may be rewarding. The susceptibility of *Bacteroides* spp. to heavy metals may be of clinical importance in specific situations. Decubitus ulcers are frequently infected with anaerobic bacteria, particularly *Bacteroides* spp. (3), and are often refractory to treatment. Chemotherapeutic agents containing heavy metals may offer a useful alternative to some of the more commonly used topical preparations.

The purpose of the present study was to examine the susceptibility of a number of strains of *Bacteroides* to several heavy metals. An additional aim of these investigations was to determine the susceptibility of *Bacteroides fragilis* to silver sulfadiazine (AgSD), the most commonly prescribed topical antimicrobial agent for burns and ulcers at our institution.

A total of 105 strains of *Bacteroides* were obtained from the Department of Microbiology Anaerobe Laboratory culture collection at the University of Western Australia. They had been identified according to the criteria of Holdeman et al. (6) and represented the *Bacteroides* spp. most commonly isolated from human infections at the Sir Charles Gairdner Hospital, Perth, Western Australia. The strains were the following: 39 *B. fragilis*, 16 *B. capillosus*, 16 *B. ruminicola*, 11 *B. fragilis* group (4 *B. distasonis*, 2 *B. ovatus*, 2 *B. thetaiotaomicron*, and 3 *B. vulgatus*) (2), 8 *B. disiens*, 6 *B. oralis*, 5 *B. multiacidus*, 3 *B. bivius*, and 1 *B. uniformis*. The cultures were maintained in prereduced supplemented brain heart infusion broth and prereduced cooked meat broth (6) at room temperature.

The following chemicals were obtained from standard commercial sources and stock solutions made at the concentrations indicated: Na_2HAsO_4 (0.1 M), $AgNO_3$ (0.1 M), $NiSO_4$ (0.1 M), $HgCl_2$ (0.01 M), $CoCl_2$ (0.1 M), $Pb(C_2H_3O_2)$ (0.1 M), $CdSO_4$ (0.1 M), and $Cr(NO_3)_3$ (0.1 M). These solutions were sterilized by filtration through 0.22-μm membrane filters (Millipore Corp., Bedford, Mass.).

Susceptibility was determined by an agar dilution method with brain heart infusion agar supplemented with 0.0005% hemin, 0.002% menadione, and 0.5% yeast extract. Plates containing 20 ml of agar and four graded final concentrations of ions were poured aerobically on the day of the experiment. The concentrations were: 0.1, 0.01, 0.001, and 0.0001 M for arsenate (As), silver (Ag), nickel (Ni), cobalt (Co), lead (Pb), cadmium (Cd), and chromium (Cr) ions and 0.01, 0.001, 0.0001, and 0.00001 M for the mercuric (Hg) ion. This range covered those concentrations usually used in the study of aerobic or facultative gram-negative bacteria (9, 13). Before use, the plates were dried at 37°C for 20 min.

The inoculum was prepared by diluting an overnight culture in supplemented brain heart infusion broth to the turbidity of a McFarland no. 1 turbidity standard and making a 1:500 dilution thereof in supplemented brain heart

TABLE 1. Susceptibility of 105 strains of *Bacteroides* to various metal ions

Metal ion	Organism[a]	Cumulative % strains susceptible at following concn (M):				
		0.00001	0.0001	0.001	0.01	0.1
As	*B. fragilis*	NT[b]	0	35	87	100
	B. fragilis group	NT	0	18	75	100
	Bacteroides spp. (other)	NT	0	50	86	100
Ag	*B. fragilis*	NT	0	40	100	100
	B. fragilis group	NT	0	81	100	100
	Bacteroides spp. (other)	NT	0	78	100	100
Ni	*B. fragilis*	NT	0	0	0	100
	B. fragilis group	NT	0	0	18	100
	Bacteroides spp. (other)	NT	0	0	21	100
Co	*B. fragilis*	NT	0	0	20	100
	B. fragilis group	NT	0	0	27	100
	Bacteroides spp. (other)	NT	0	0	36	100
Pb	*B. fragilis*	NT	0	0	4	100
	B. fragilis group	NT	0	0	18	100
	Bacteroides spp. (other)	NT	0	0	14	100
Cd	*B. fragilis*	NT	0	4	100	100
	B. fragilis group	NT	0	18	100	100
	Bacteroides spp. (other)	NT	0	14	100	100
Cr	*B. fragilis*	NT	0	0	0	100
	B. fragilis group	NT	0	0	0	100
	Bacteroides spp. (other)	NT	0	0	0	100
Hg	*B. fragilis*	0	0	100	100	NT
	B. fragilis group	0	0	100	100	NT
	Bacteroides spp. (other)	0	0	100	100	NT

[a] Numbers tested: *B. fragilis*, 39; *B. fragilis* group, 11; *Bacteroides* spp. (other), 55.
[b] NT, Not tested.

infusion broth. The agar plates were inoculated with a Steer's replicator, resulting in a final inoculum of approximately 10^3 bacteria per spot. The plates were incubated in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) at 37°C for 24 h. After incubation, the minimum inhibitory concentration was determined as the lowest concentration of metal ion preventing growth. For the purpose of defining resistance, strains which were inhibited by 0.01 M As; 0.001 M Ag, Ni, Co, Pb, Cd, and Cr; and 0.0001 M Hg were regarded as resistant. These concentrations have been used in previous studies of facultative bacteria (9, 13). A strain of *Escherichia coli* (NCTC 10418) known to be susceptible to these concentrations of ions and an agar plate containing no metal ions were used as controls.

A total of 25 strains of *B. fragilis* were tested against AgSD by the following method. The surface of a plate containing brain heart infusion agar supplemented with 0.0005% hemin, 0.002% menadione, and 0.5% yeast extract was flooded with an inoculum prepared as described above. The excess was removed, and the agar plate was allowed to dry. With a sterile cork borer, a 9-mm-diameter hole was punched in the center of the agar plate. With an Oxford sampler, 100 μl of a 0.1% AgSD suspension was placed in the well, and the plate was incubated in a GasPak jar overnight at 37°C. Any zone of inhibition was considered to indicate susceptibility.

The *Bacteroides* spp. tested were conveniently divided into *B. fragilis*, *B. fragilis* group, and other *Bacteroides* spp. The cumulative percentages of strains susceptible to various concentrations of metal ions for these groups are presented in Table 1. Overall, the results were similar for all three groups. However, strains of *B. fragilis* were significantly more resistant than the other two groups to Ag ($P < 0.0005$, χ^2) and slightly more resistant to Ni, Co, Pb, and Cd. It is interesting to note that the strains inhibited by 0.01 M Pb were also inhibited by 0.001 M Cd, suggesting, possibly, a common type of resistance. All strains of *Bacteroides* tested were resistant to multiple metals ions: 7 were resistant to five metals ions (6%), 30 were resistant to six metal ions (28.6%), 60 were resistant to seven metals ions (57.1%), and 8 were resistant to all eight metal ions (7.6%). The frequencies of resistance for all strains for each metal ion tested were as follows: As, 25%; Ag, 48%; Ni, 100%; Co, 100%; Pb, 100%; Cd, 92%; Cr, 100%; and Hg, 100%. All 25 strains of *B. fragilis* tested against AgSD were susceptible.

A wide range of concentrations was tested because of the paucity of information on the susceptibility of *Bacteroides* spp. to heavy metals. It was hoped that an inhibitory concentration would be obtained which could be used as a guide for further investigations and that some comparisons with previously reported studies of facultative bacteria (9, 13) could be made. Indeed, from the results it would appear that *Bacteroides* spp. are far more resistant than their facultative counterparts to the action of heavy metals.

It is surprising, therefore, that reports in older literature suggested that some anaerobic bacteria were susceptible to heavy metals and metalloids (4). Chrysotherapy was apparently quite common some years ago. Christiaens et al. (cited in reference 4) reported a case with widespread infection due to *Fusiformis fusiformis* which responded to therapy with gold thiosulfate. Arsphenamines were also once widely used both systemically and topically in the treatment of so-called fusospirochaetal infections (11). Although both these agents have lost favor for modern day therapeutic regimens, our results indicate that As ions were the most active of the chemicals tested, with 75% of *Bacteroides* spp. susceptible.

Of particular importance from a clinical viewpoint is resistance to silver and mercury. The use of topical $AgNO_3$ and, occasionally, preparations containing mercury has been widespread in the management of burns. Transferable silver resistance has been demonstrated in *Salmonella typhimurium* (8), and there is evidence for its plasmid location in *Enterobacter cloacae* (1, 12). Transmissible plasmids in a variety of gram-negative bacilli, including *E. coli*, *S. typhimurium*, and *Pseudomonas* sp., have been shown to mediate mercury resistance (13, 15). Several studies have now shown that *Bacteroides* spp. contain transferable plasmids (7, 17, 20). Whether or not there is a relationship between resistance to some heavy metals and the presence of plasmids in *Bacteroides* spp. remains to be investigated, although Wallace et al. (19) could find no correlation in a small number of strains studied. If resistance to some heavy metals were plasmid mediated, one would expect to find a bimodal distribution of susceptibility. This is not apparent from our data; an alternative and possibly more acceptable interpretation is that heavy metal resistance in *Bacteroides* spp. is intrinsic rather than plasmid borne.

With the high incidence and levels of Ag resistance in *B. fragilis*, it was surprising to find that all 25 strains tested were susceptible to AgSD. The mechanism of AgSD action has only been investigated with *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and the SD component accounted for little of the activity of the combination (5). Riley (10) showed that sulfonamides were effective against *B. fragilis* in vitro, and possibly the SD component is more active against *Bacteroides* spp. It would be interesting to compare the efficacy of AgSD with that of a topical sulfonamide for the treatment of ulcers infected with anaerobic bacteria. If successful, this would be a far less expensive form of treatment for the very common problem of decubitus ulcers.

LITERATURE CITED

1. **Annear, D. I., B. J. Mee, and M. Bailey.** 1976. Instability and linkage of silver resistance, lactose fermentation, and colony structure in *Enterobacter cloacae* from burn wounds. J. Clin. Pathol. **29:**441–443.
2. **Cato, E. P., and J. L. Johnson.** 1976. Reinstatement of species rank for *Bacteroides fragilis*, *B. ovatus*, *B. distasonis*, *B. thetaiotaomicron*, and *B. vulgatus*: designation of neotype strains for *Bacteroides fragilis* (Veillon and Zuber) Castellani and Chalmers and *Bacteroides thetaiotaomicron* (Distaso) Castellani and Chalmers. Int. J. Syst. Bacteriol. **26:**230–237.
3. **Daltrey, D. C., B. Rhodes, and J. G. Chattwood.** 1981. Investigation into the microbial flora of healing and non-healing decubitus ulcers. J. Clin. Pathol. **34:**701–705.
4. **Finegold, S. M.** 1977. Anaerobic bacteria in human disease. Academic Press, Inc., New York.
5. **Fox, C. L., and S. M. Modak.** 1974. Mechanism of silver sulfadiazine action on burn wound infections. Antimicrob. Agents Chemother. **5:**582–588.
6. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual. Virginia Polytechnic Institute and State University, Blacksburg.
7. **Mancini, D., and R. J. Behme.** 1977. Transfer of multiple antibiotic resistance from *Bacteroides fragilis* to *Escherichia coli*. J. Infect. Dis. **136:**597–600.
8. **McHugh, G. L., R. C. Moellering, C. C. Hopkins, and M. N. Swartz.** 1975. *Salmonella typhimurium* resistant to silver nitrate, chloramphenicol, and ampicillin. Lancet **i:**235–239.
9. **Nakahara, H., T. Ishikawa, Y. Sarai, I. Kondo, and H. Kozukue.** 1978. Survey of resistance to metals and antibiotics in clinical isolates of *Klebsiella pneumoniae* in Japan. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **240:**22–29.
10. **Riley, T. V.** 1981. Agar dilution susceptibility of *Bacteroides* spp. to sulfamethoxazole and trimethoprim: correlation with a disk diffusion technique. Antimicrob. Agents Chemother. **20:**731–735.
11. **Rosebury, T., G. Foley, and F. L. Right.** 1939. Effects of neoarsphenamine and sulpharsphenamine on experimental fuso-spirochetal infection in guinea pigs. J. Infect. Dis. **65:**291–294.
12. **Rosenkranz, H. S., J. E. Coward, T. J. Wlodkowski, and H. S. Carr.** 1974. Properties of silver sulfadiazine-resistant *Enterobacter cloacae*. Antimicrob. Agents Chemother. **5:**199–201.
13. **Smith, D. H.** 1967. R factors mediate resistance to mercury, nickel, and cobalt. Science **156:**1114–1115.
14. **Stiffer, P. W., R. Keller, and N. Traub.** 1974. Isolation and characterization of several cryptic plasmids from clinical isolates of *Bacteroides fragilis*. J. Infect. Dis. **130:**544–548.
15. **Summers, A. O., and E. Lewis.** 1973. Volatilization of mercury chloride by mercury-resistant plasmid-bearing strains of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. J. Bacteriol. **113:**1070–1072.
16. **Summers, A. O., and S. Silver.** 1978. Microbial transformation of metals. Annu. Rev. Microbiol. **32:**637–672.
17. **Tally, F. P., D. R. Syndman, S. L. Gorbach, and M. H.

Malamy. 1979. Plasmid-mediated transferable resistance to clindamycin and erythromycin in *Bacteroides fragilis*. J. Infect. Dis. **139**:83–88.

18. **Tinnell, W. H., and F. L. Macrina.** 1976. Extra chromosomal elements in a variety of strains representing the *Bacteroides fragilis* group of organisms. Infect. Immun. **14**:955–964.
19. **Wallace, B. L., J. E. Bradley, and M. Rogolsky.** 1981. Plasmid analysis in clinical isolates of *Bacteroides fragilis* and other *Bacteroides* species. J. Clin. Microbiol. **14**:383–388.
20. **Welch, R. A., K. R. Jones, and F. L. Macrina.** 1979. Transferable lincosamide-macrolide resistance in *Bacteroides*. Plasmid **2**:261–268.